

Chrysosporium lucknowense cellulase production platform for applications in biorefineries

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Introduction

Dyadic International, Inc. owns and develops the fungus *Chrysosporium lucknowense* C1 as a platform for the hyper production of a broad variety of enzymes. The C1 genome was sequenced and annotated, revealing numerous potential product opportunities. These include, amongst others, versatile enzymes for the production of biofuels from renewable (non-starch) feed stocks. Here, we describe how this knowledge was used to develop C1 strains, which produce tailor made enzyme mixtures for the efficient saccharification of plant biomass.

C1 genome sequencing project

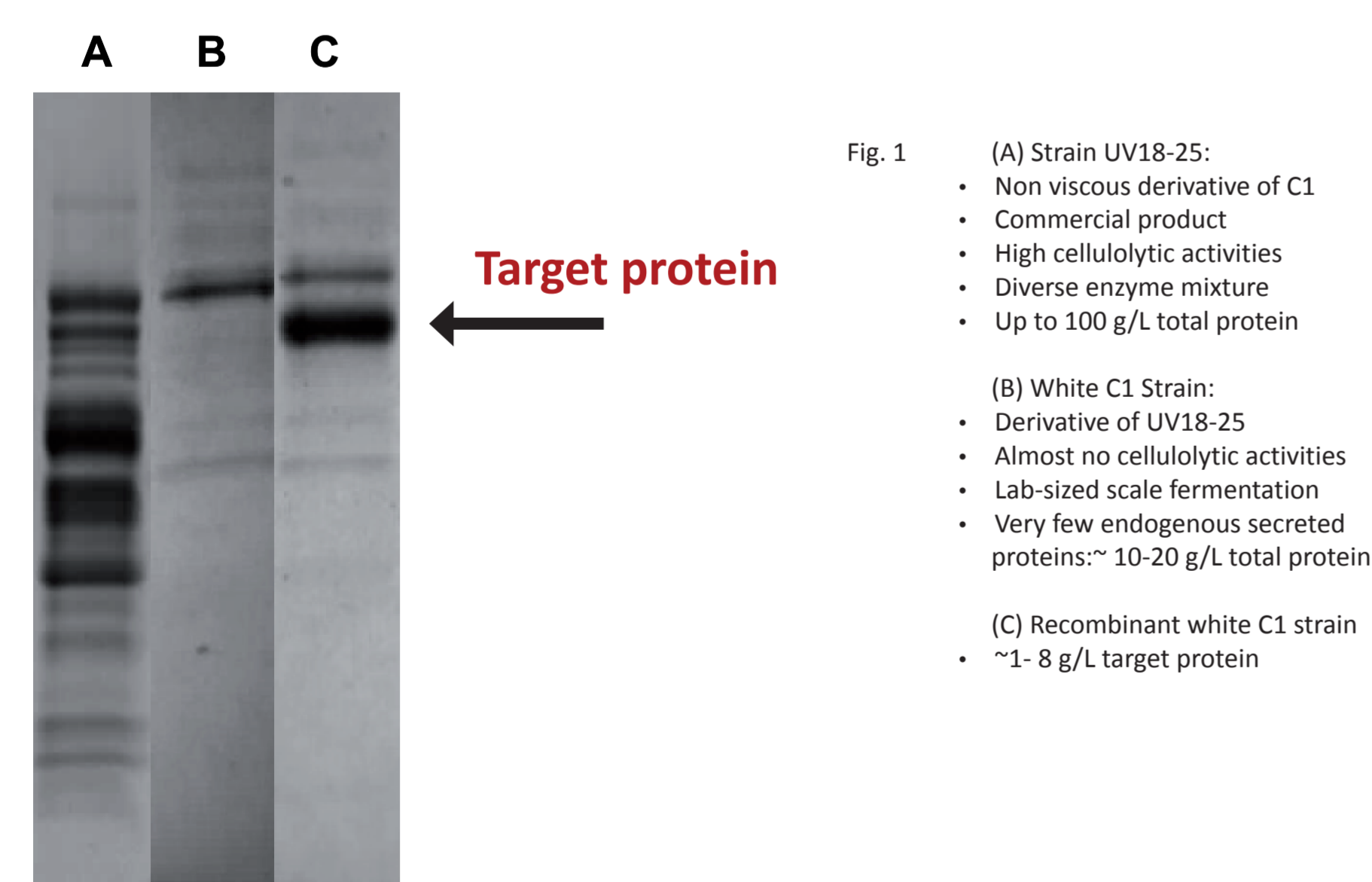
The sequencing and automated annotation of the C1-genome has been completed. The C1 genome size is about 38 million base pairs and contains approximately 11,000 (putative) genes. The knowledge obtained facilitates additional host strain improvements and revealed numerous potential product opportunities.

Table 1. Comparison of the C1, *Aspergillus niger* and *Trichoderma reesei* genomes with respect to the number of a subset of putative (hemi-)cellulase encoding genes. GH, CAZy glycoside hydrolase family number. CE, CAZy carbohydrate esterase family number.

	C1	<i>A. niger</i> *	<i>T. reesei</i> **
Cellulases (GH3, 5, 6, 7, 12)	~ 29	~ 35	~ 26
Cellulases (GH61)	~ 24	~ 7	~ 9
Cellulose binding domains (CBM1)	~ 46	~ 8	~ 11
Xylanases	~ 11	~ 5	~ 5
Arabinofuranosidases/arabinases	~ 14	~ 13	~ 3
Esterases (Axe, Fae)	~ 10	~ 10	~ 2

C1's biomass converting potential

C1-genome mining revealed an impressive hydrolytic potential. For example a large set of putative (hemi-) cellulase encoding genes have been discovered. Remarkable differences were observed when compared to other fungi. An example of a subset of these genes is given in Table 1. The molecular tools developed and the isolation of low background C1 strains allowed the production of high amounts of individual (hemi-) cellulases (Fig. 1). Over 60 C1 (hemi-) cellulase encoding genes have been over-expressed in C1. In an ongoing effort these enzymes are being purified and characterized.



Characterization of artificial enzyme mixes

Existing commercial crude enzyme preparations of C1 (NCE-L600) performed poorly in the saccharification of ligno-cellulosic substrates (Fig. 2). In order to improve the C1 preparation, several cellulolytic enzymes that are expressed by this strain were overexpressed in the white C1 strain and purified. Different mixtures of these purified C1 cellobiohydrolases, endoglucanases, β -glucosidase and a xylanase were subsequently tested for saccharification performance on the same pretreated ligno-cellulosic substrate.

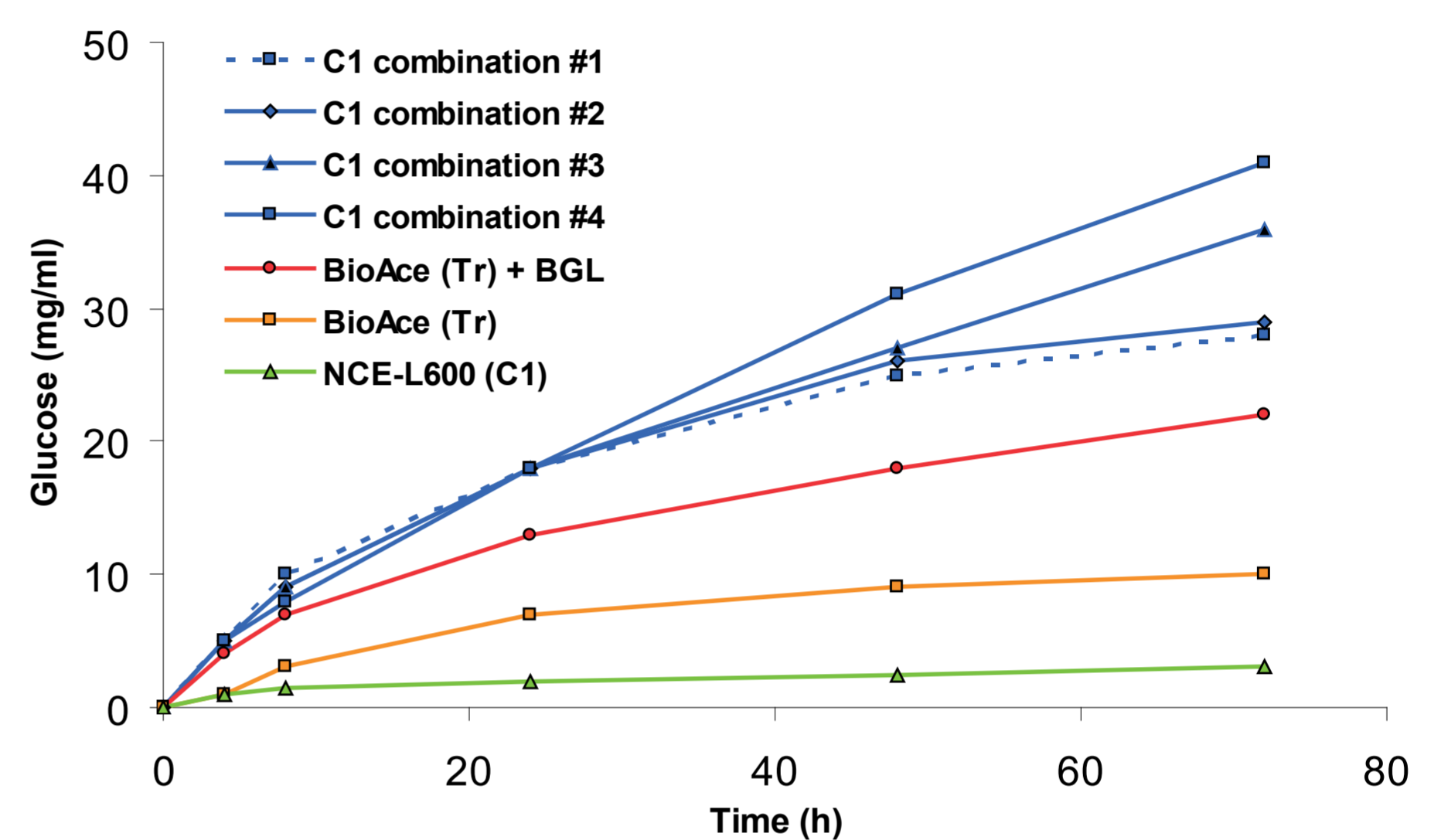


Fig 2. Kinetics of hydrolysis of pretreated Douglas fir wood (50 mg/ml) by different combinations of purified C1 enzymes and commercial crude cellulase preparations from C1 and *Trichoderma reesei* (Tr) each at protein loading of 0.5 mg/ml, 50 °C, pH 5.0(1). (C1 combination (mg/ml) #1: 0.2 CBH Ia, 0 CBH Ib, 0.2 CBH IIb, 0.08 EG II, 0.02 BGL; #2: 0.2 CBH Ia, 0.2 CBH IIb, 0.07 EG II, 0.02 BGL, 0.01 Xyl II; #3: 0.2 CBH Ia, 0.2 CBH IIb, 0.04 EG II, 0.04 EG V, 0.02 BGL; #4: 0.1 CBH Ia, 0.1 CBH Ib, 0.2 CBH IIb, 0.03 EG II, 0.04 EG V, 0.02 BGL, 0.01 Xyl II.)

This showed that the tested mixtures were significantly more effective on this substrate than the enzyme mixture naturally produced by the C1 strain i.e. the commercial enzyme mixtures (Fig. 2). In general, these type of mixing and matching studies revealed which enzymes were insufficiently present in the ligno-cellulose converting enzyme mixture produced by C1 strains. By means of overexpressing specific C1 genes, these enzyme activities were supplemented resulting in C1 strains that produce optimized enzyme mixtures for efficient saccharification of different types of plant biomass (Fig 3). The availability of a single strain producing the most suitable mix of enzymes allows for cost effective production.

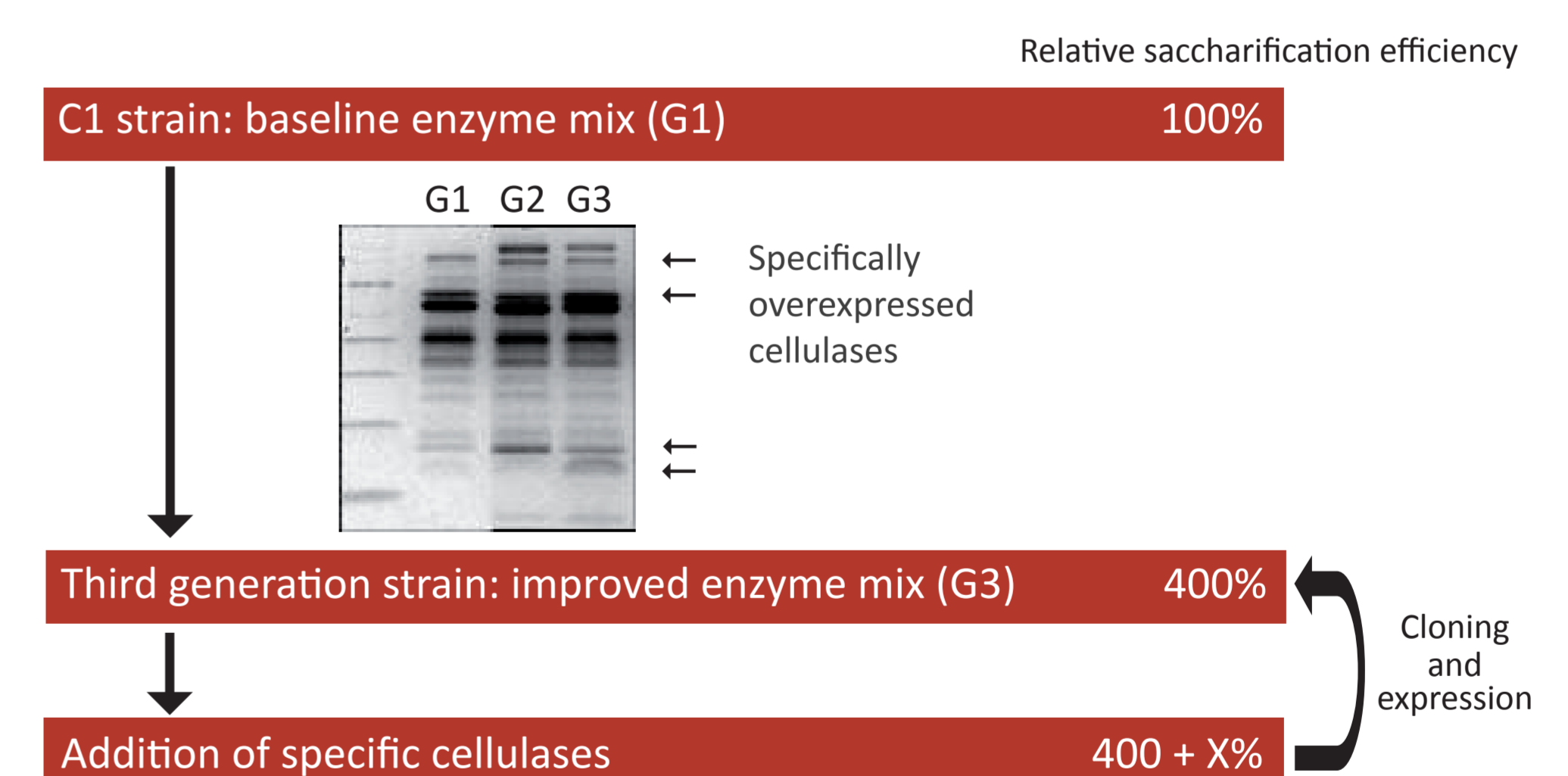


Fig 3. General approach to obtain improved C1 strains producing optimal enzyme mixes for saccharification. G1, G2 and G3 stand for the subsequent C1 strain generations.

Conclusion

C. lucknowense C1 is a rich source of numerous (new) enzymes for the conversion or modification of (hemi-) cellulosic materials. C1 strains producing tailored enzyme mixtures for biorefinery applications are continuously being constructed based on characterization of new individual enzymes and kinetic analyses of artificial enzyme mixes. This approach has already resulted in highly efficient enzyme mixes produced by one C1-strain. Given the progress made and the great (hemi-) cellulolytic potential still to be explored and exploited, the outlook for an economically sound application of C-enzymes in biorefinery processes is excellent.